

Supplementary Information for

**AMPA receptor anchoring at CA1 synapses is determined by N-terminal domain and TARP  $\gamma 8$  interactions**

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**Containing:**

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Supplementary Figures 1-7

**Supplementary Table 1: Dual Cell Synaptic Current Properties - GluA2Q data**

Construct	Rectification Index			
	Untrans.	Transf.	n (pairs)	p*
GluA2Q	0.61 ± 0.05	0.22 ± 0.03	6	0.0009
GluA2Q ΔNSF	0.62 ± 0.04	0.19 ± 0.028	11	<0.0001
GluA2Q ΔPDZ	0.60 ± 0.08	0.24 ± 0.04	8	0.0008
GluA2Q A1ICD	0.49 ± 0.05	0.17 ± 0.03	12	<0.0001
GluA2Q CTD-null	0.62 ± 0.04	0.19 ± 0.03	15	<0.0001
GluA2Q ΔNTD	0.60 ± 0.04	0.21 ± 0.03	9	<0.0001
GluA2Q ΔNTD ΔNSF	0.56 ± 0.05	0.38 ± 0.03	10	0.013
GluA2Q ΔNTD ΔPDZ	0.57 ± 0.04	0.27 ± 0.03	12	<0.0001
GluA2Q ΔNTD A1ICD	0.56 ± 0.05	0.41 ± 0.03	9	0.018
GluA2Q ΔNTD CTD-null	0.61 ± 0.02	0.15 ± 0.02	15	<0.0001

Construct	AMPA EPSC Amplitudes (pA)			
	Untrans.	Transf.	n (pairs)	p*
GluA2Q	37.4 ± 10.2	54.8 ± 10.2	8	0.0078
GluA2Q ΔNSF	34.8 ± 3.9	48.2 ± 7.5	11	0.024
GluA2Q ΔPDZ	23.1 ± 2.9	34.7 ± 5.3	9	0.0039
GluA2Q A1ICD	39.1 ± 8.0	54.6 ± 9.1	12	0.043
GluA2Q CTD-null	53.1 ± 6.3	65.7 ± 8.5	20	0.030
GluA2Q ΔNTD	40.6 ± 4.1	21.8 ± 2.2	14	0.0001
GluA2Q ΔNTD ΔNSF	37.6 ± 3.2	29.4 ± 4.5	11	0.024
GluA2Q ΔNTD ΔPDZ	91.4 ± 15.5	65.8 ± 11.0	12	0.016
GluA2Q ΔNTD A1ICD	38.6 ± 5.0	31.7 ± 6.1	9	0.049
GluA2Q ΔNTD CTD-null	50.0 ± 4.5	30.2 ± 3.6	15	0.0002

\* Statistical analysis - Rectification Index: two-tailed paired t-test; EPSC amplitudes: two-tailed Wilcoxon matched-pairs signed rank test.

**Supplementary Table 2: Dual Cell Synaptic Current Properties - GluA1 data**

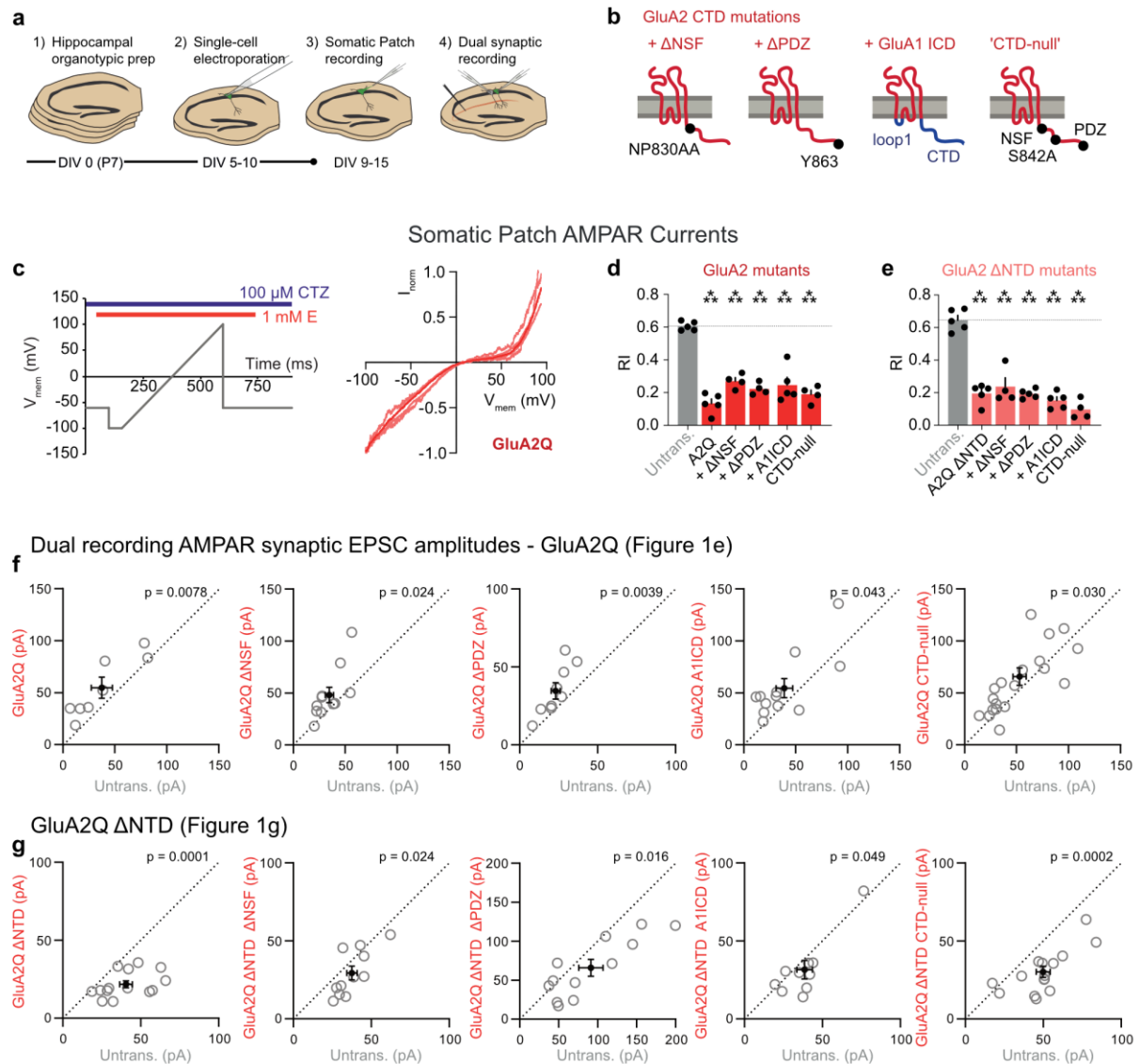
Construct	Rectification Index			
	Untrans.	Transf.	n (pairs)	p*
GluA1	0.53 ± 0.03	0.37 ± 0.03	7	0.0058
GluA1 ΔPDZ	0.60 ± 0.04	0.38 ± 0.04	13	<0.0001
GluA1 ΔPDZ +tCKII	0.58 ± 0.06	0.41 ± 0.05	12	0.012
GluA1 CTD-null	0.58 ± 0.04	0.45 ± 0.04	17	0.0016
GluA1 + TTX incubation	0.64 ± 0.07	0.31 ± 0.03	7	0.0082
GFP-GluA1	0.60 ± 0.04	0.54 ± 0.04	16	0.086
GluA1 ΔNTD	0.57 ± 0.07	0.53 ± 0.05	9	0.54
GluA1 ΔNTD ΔPDZ	0.52 ± 0.05	0.49 ± 0.04	9	0.41
GluA1 ΔNTD +tCKII	0.56 ± 0.05	0.45 ± 0.05	10	0.0083
GluA1 ΔNTD ΔPDZ + tCKII	0.63 ± 0.03	0.60 ± 0.05	8	0.70

Construct	AMPA EPSC Amplitudes (pA)			
	Untrans.	Transf.	n (pairs)	p*
GluA1	24.5 ± 1.4	19.6 ± 1.8	7	0.078
GluA1 ΔPDZ	60.8 ± 7.2	56.4 ± 8.2 pA	14	0.30
GluA1 ΔPDZ +tCKII	50.8 ± 9.0	106.2 ± 16.0	8	0.0078
GluA1 CTD-null	46.8 ± 5.8	37.9 ± 3.8	17	0.38
GluA1 + TTX incubation	72.3 ± 12.7	52.2 ± 5.6	8	0.15
GFP-GluA1	65.3 ± 5.7	49.8 ± 5.4	13	0.13
GluA1 ΔNTD	29.5 ± 4.9	19.1 ± 2.3	9	0.055
GluA1 ΔNTD ΔPDZ	59.2 ± 6.1	47.5 ± 4.4	9	0.30
GluA1 ΔNTD +tCKII	49.9 ± 6.8	43.1 ± 12.0	9	0.13
GluA1 ΔNTD ΔPDZ + tCKII	21.7 ± 2.7	38.8 ± 4.7	16	0.0016

\* Statistical analysis - Rectification Index: two-tailed paired t-test; EPSC amplitudes: two-tailed Wilcoxon matched-pairs signed rank test.

**Supplementary Table 3: List of Primers used for plasmid generation**

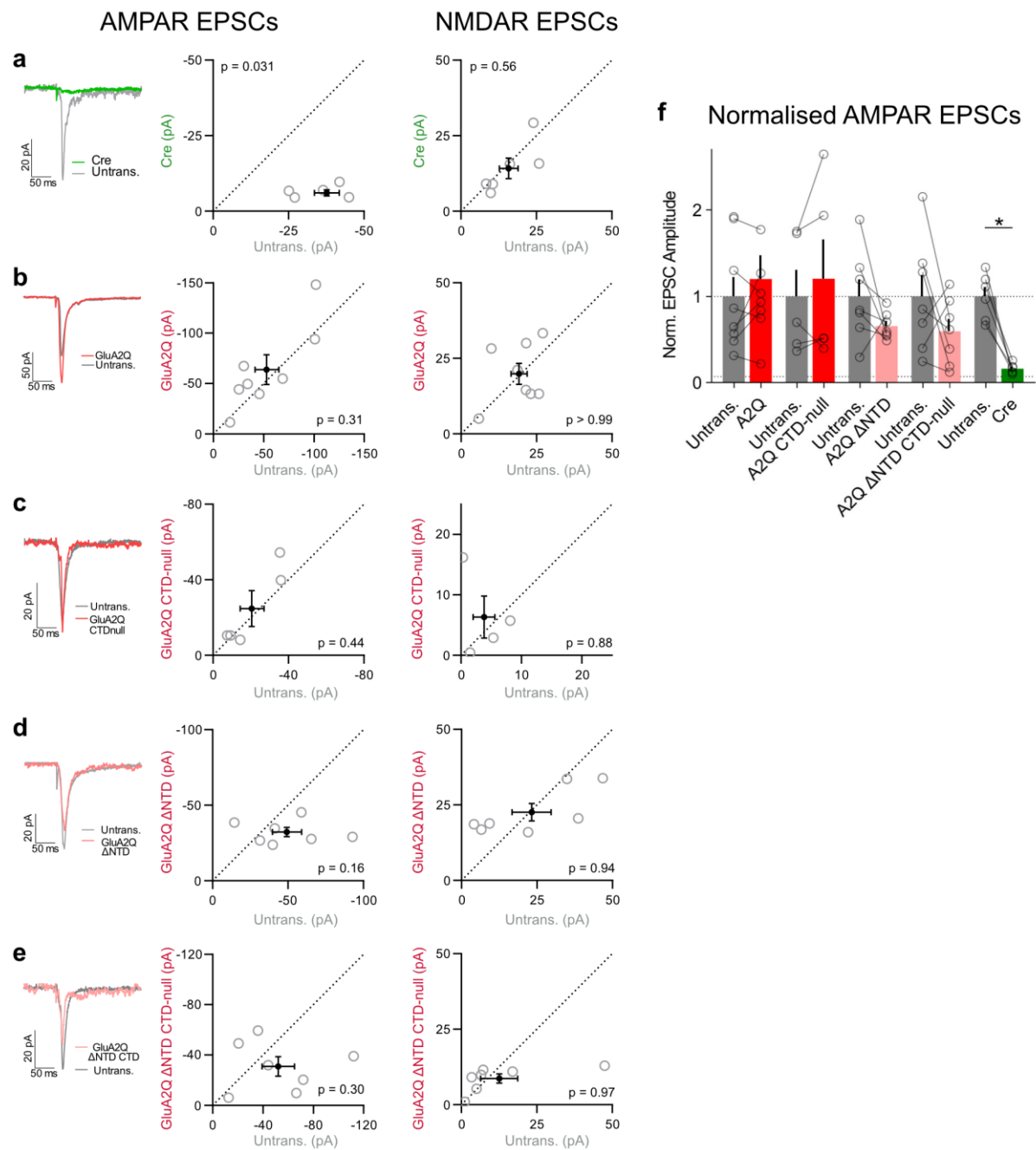
<b>Name</b>	<b>Sequence</b>	<b>Notes</b>
scFv-Clasp light chain forward primer	5'-TAGTAGTAACCGGTCATATGGAAGTGAAG-TTGGTTGAATCTG-3'	inserting AgeI site
scFv-Clasp light chain reverse primer	5'-TAGTAGTAGGTACCCTTAGCCTCTATGGC-ATCCAGG-3'	Inserting KpnI site
scFv-Clasp heavy chain forward primer	5'-TAGTAGTAACCGGTATGGACATCGAATTG-ACTCAGTCTCC-3'	inserting AgeI site
scFv-Clasp heavy chain reverse primer	5'-TAGTAGTATGTACATTACTTAGCCTCTAT-GGCATCCA-GG-3'	Inserting Bsp1407I site



**Supplementary Figure 1. Somatic and Synaptic recordings on GluA2Q expression.** **a** Approach for analysis of mutant AMPAR constructs. **b** Construct schematics depicting the locations of CTD mutations on GluA2 ΔNTD. Peptide topology is displayed relative to the membrane (grey). **c** Somatic AMPAR currents demonstrate surface trafficking for GluA2 mutations by rectification index measurements. Protocol for surface rectification measurement by glutamate (E) and cyclothiazide (CTZ) application during a membrane voltage ramp (left). Representative glutamate gated currents for GluA2 overexpressing neurons (right, individual cells - light, average response - bold), normalised to -100 mV amplitude. **d** Somatic patch rectification index on overexpression of CTD mutation constructs of GluA2Q (Untrans.:  $0.61 \pm 0.01$ ,  $n = 5$ ; GluA2Q:  $0.13 \pm 0.03$ ,  $n = 5$ ; GluA2Q ΔNSF:  $0.27 \pm 0.02$ ,  $n = 4$ ; GluA2Q ΔPDZ:  $0.22 \pm 0.02$ ,  $n = 4$ ; GluA2Q A1ICD:  $0.25 \pm 0.05$ ,  $n = 5$ ; GluA2Q CTD-null:  $0.19 \pm 0.03$ ,  $n = 4$ ; [ $F(5, 21) = 36.38$ ,  $p < 0.0001$ ], One-way ANOVA with Dunnett's multiple comparisons test, bars

represent mean values), and **e**, GluA2Q  $\Delta$ NTD (Untrans.:  $0.65 \pm 0.03$ ,  $n = 5$ ; GluA2Q  $\Delta$ NTD:  $0.20 \pm 0.03$ ,  $n = 5$ ; GluA2Q  $\Delta$ NTD  $\Delta$ NSF:  $0.24 \pm 0.05$ ,  $n = 4$ ; GluA2Q  $\Delta$ NTD  $\Delta$ PDZ:  $0.19 \pm 0.01$ ,  $n = 5$ ; GluA2Q  $\Delta$ NTD A1ICD:  $0.15 \pm 0.02$ ,  $n = 5$ ; GluA2Q  $\Delta$ NTD CTD-null:  $0.10 \pm 0.03$ ,  $n = 4$ ; [ $F(5, 22) = 46.22$ ,  $p < 0.0001$ ], One-way ANOVA with Dunnett's multiple comparisons test, bars represent mean values).

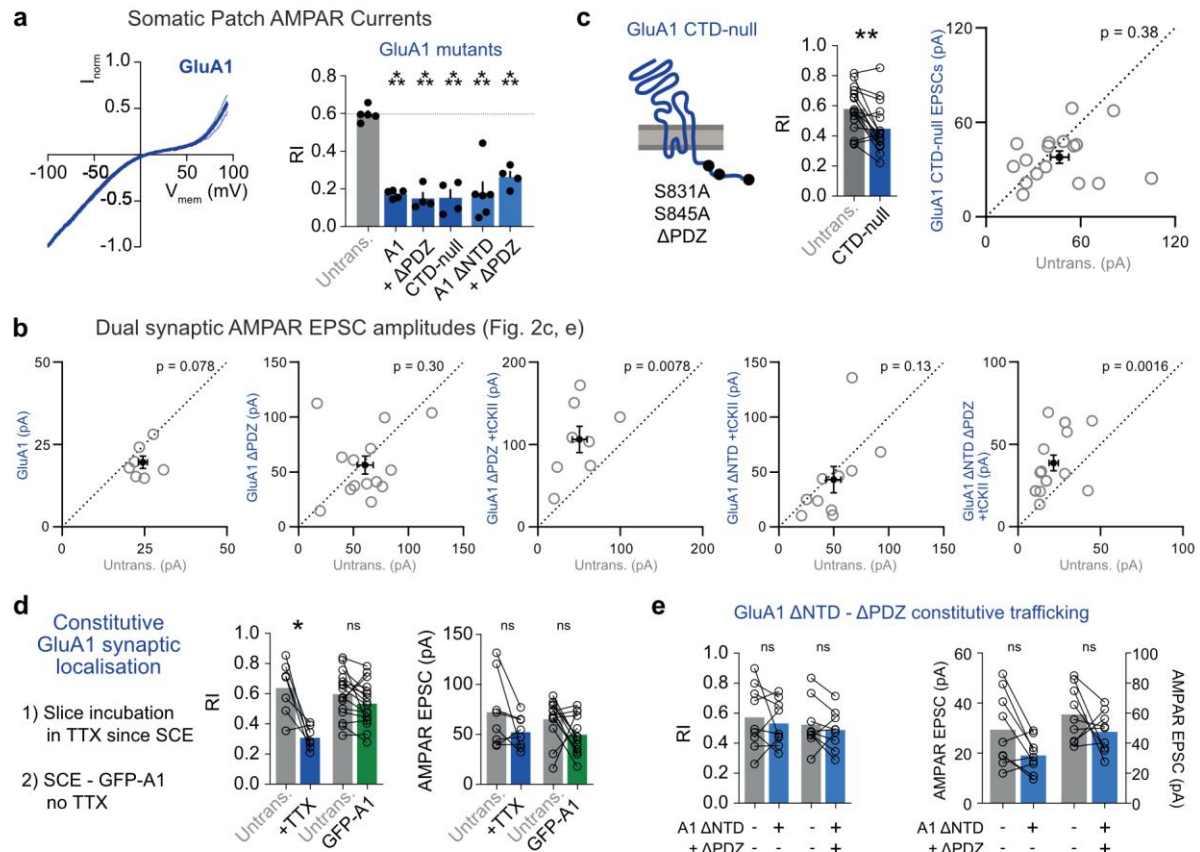
**f-g** Scatters of dual synaptic EPSC amplitudes for normalized data presented in **Figure 1e** and **1g**. Filled circles represent mean  $\pm$  SEM. Data values are presented in **Supplementary Table 1**. Source data are provided as a Source Data file.



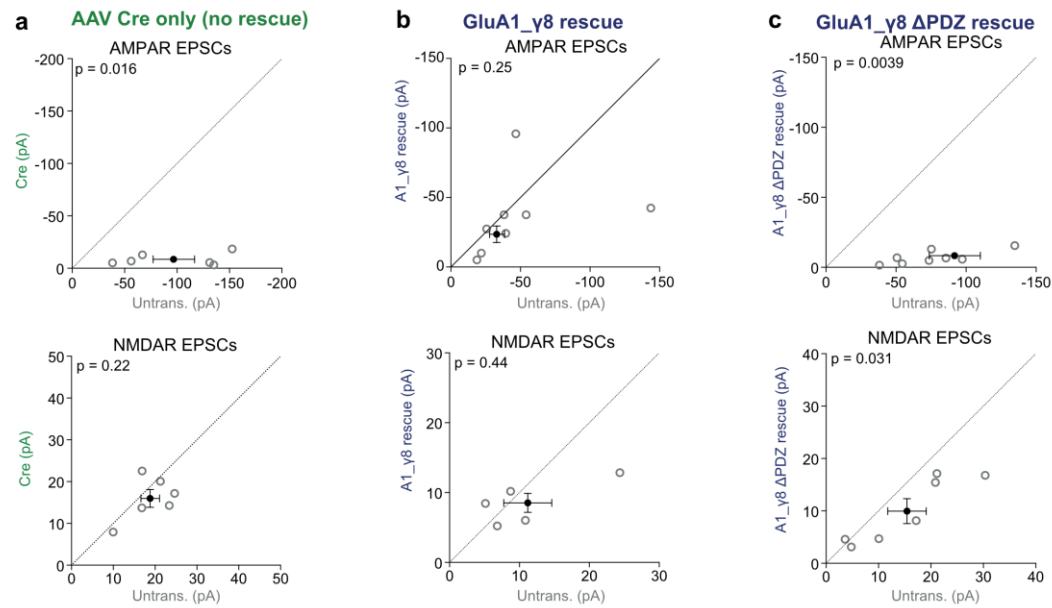
**Supplementary Figure 2. Recovery of AMPAR-null transmission by CTD-null receptors. a** Expression of Cre-recombinase in Gria1-3fl tissue abolished AMPAR, but not NMDAR synaptic transmission (AMPA EPSCs (pA) - Untransduced (Untrans):  $37.6 \pm 4.1$  pA, Cre:  $6.1 \pm 0.9$  pA,  $n=6$  pairs,  $p=0.031$ . NMDAR EPSCs (pA) – Untrans.:  $15.8 \pm 3.1$  pA, Cre:  $1.2 \pm 3.4$  pA,  $n=6$ ,  $p=0.56$ ). **b-e** GluA2Q and GluA2Q  $\Delta$ NTD with and without CTD interaction site mutagenesis (CTD-null) can rescue AMPAR EPSCs, demonstrating no requirement for the CTD in synaptic anchoring (GluA2Q - Untrans.:  $53.1 \pm 11.9$  pA, Rescue:  $63.8 \pm 14.7$  pA,  $n = 8$  pairs,  $p=0.031$ . GluA2Q CTD-null – Untrans.:  $20.6 \pm 6.3$  pA, Rescue:  $24.7 \pm 9.4$  pA,  $n = 5$  pairs,  $p=0.44$ . GluA2Q  $\Delta$ NTD – Untrans.:  $49.2 \pm 9.7$  pA, Rescue:  $32.3$

$\pm 2.9$  pA,  $n = 7$  pairs,  $p=0.16$ . GluA2Q  $\Delta$ NTD CTD-null – Untrans.:  $52.1 \pm 13.0$  pA, Rescue:  $30.9 \pm 7.5$  pA,  $n = 7$  pairs,  $p=0.30$ ), while NMDAR EPSCs were unaffected (GluA2Q - Untrans.:  $19.2 \pm 2.6$  pA, Rescue:  $19.9 \pm 3.5$  pA,  $n = 8$  pairs,  $p=0.56$ . GluA2Q CTD-null – Untrans.:  $3.8 \pm 1.8$  pA, Rescue:  $6.3 \pm 3.5$  pA,  $n = 4$  pairs,  $p>0.99$ . GluA2Q  $\Delta$ NTD – Untrans.:  $23.2 \pm 6.5$  pA, Rescue:  $22.6 \pm 2.9$  pA,  $n = 7$  pairs,  $p=0.88$ . GluA2Q  $\Delta$ NTD CTD-null – Untrans.:  $12.6 \pm 6.1$  pA, Rescue:  $8.7 \pm 1.6$  pA,  $n = 7$  pairs,  $p=0.97$ ). Filled circles represent mean  $\pm$  SEM. **f** Data normalized to Untransfected EPSC amplitudes demonstrates that CTD modification does not strongly influence rescue by either GluA2Q or GluA2Q  $\Delta$ NTD. Bars represent mean values + SEM. For n-numbers and p-values see panels a-e, \* indicates  $p<0.05$ . All statistical analysis (**S2 a-f**) were performed with a Wilcoxon matched-pairs signed rank test. Source data are provided as a Source Data file.



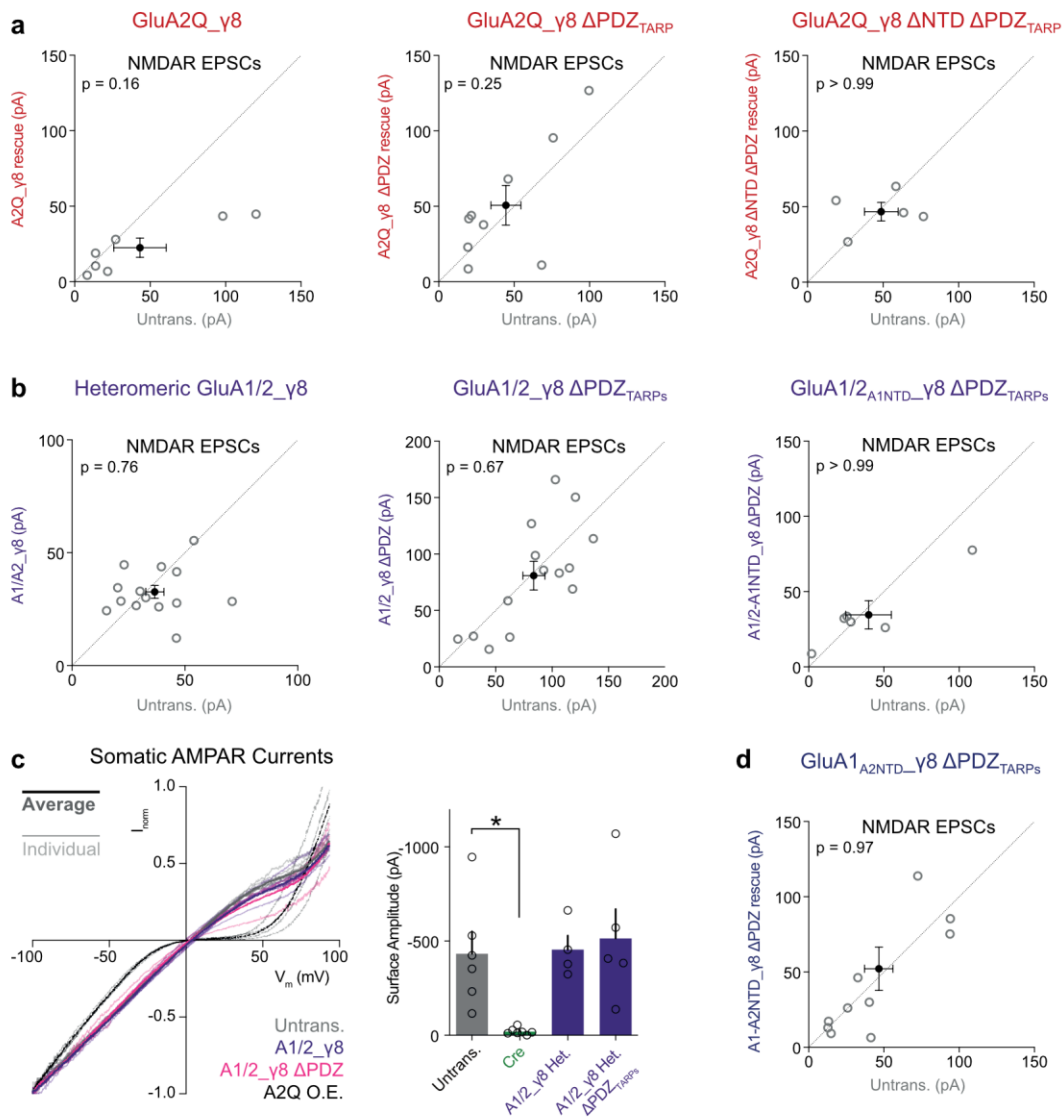


**Supplementary Figure 3. Somatic and Synaptic recordings on expression of GluA1.** **a** Somatic AMPAR currents demonstrate surface trafficking for GluA1 mutations by rectification index measurements GluA1 (Untrans.:  $0.60 \pm 0.02$ ,  $n = 5$ ; GluA1:  $0.17 \pm 0.01$ ,  $n = 5$ ; GluA1  $\Delta$ PDZ:  $0.15 \pm 0.03$ ,  $n = 4$ ; GluA1 CTD-null:  $0.15 \pm 0.04$ ; GluA1  $\Delta$ NTD:  $0.18 \pm 0.06$ ,  $n = 6$ ; GluA1  $\Delta$ NTD  $\Delta$ PDZ:  $0.26 \pm 0.03$ ,  $n = 4$ ; [ $F(5, 22) = 21.73$ ,  $p < 0.0001$ ], One-way ANOVA with Dunnett's multiple comparisons test). **b** Synaptic EPSC amplitudes for normalized data in **Figures 2c** and **2e**. **c** Schematic and synaptic recordings demonstrating synaptic localisation of GluA1 CTD-null receptors (RI: two-tailed paired t-test,  $p = 0.0016$ , AMPAR EPSCs: two-tailed Wilcoxon matched-pairs signed rank test,  $p = 0.38$ ). **d** GluA1 constitutive synaptic localization does not require ongoing slice activity, as RI is altered even which activity is blocked by  $1 \mu\text{M}$  TTX application from the day of transfection. Synaptic localization of GluA1 is prevented by EGFP-tagging at the N-terminus. **e** GluA1  $\Delta$ NTD does not contribute to synaptic transmission regardless of PDZ<sub>GluA1</sub> interactions (Wilcoxon matched-pairs signed rank test). Data values are presented in **Supplementary Table 2**. \* indicates  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  and <sup>ns</sup> specifies no significance. Bars in panels a, c-e represent mean values, filled circles in b-c represent mean  $\pm$  SEM. Source data are provided as a Source Data file.



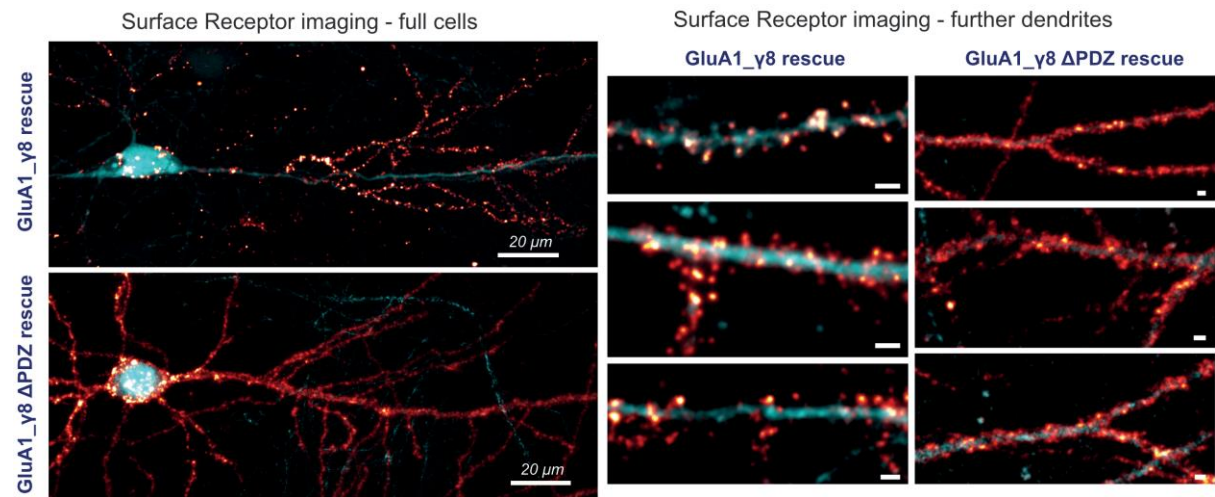
**Supplementary Figure 4. Supporting data for knockout and rescue of GluA1\_ γ8 receptors. a-c**

Scatter graphs of synaptic AMPAR and NMDAR EPSC amplitudes between Cre-transduced and untransduced (Untrans.) neurons (**a** - AMPAR EPSCs - Untrans.:  $-86.5 \pm 19.6$  pA, Cre:  $-7.9 \pm 2.2$  pA,  $n = 7$  pairs,  $p=0.016$ ; NMDAR EPSCs - Untrans.:  $22.7 \pm 5.4$  pA, Cre:  $18.3 \pm 3.3$  pA,  $n = 6$  pairs,  $p=0.22$ ), control and GluA1\_γ8 rescued pairs (**b** - AMPAR EPSCs - Untrans.:  $-48.8 \pm 14.3$  pA, GluA1\_γ8 rescue:  $-35.1 \pm 9.9$  pA,  $n = 8$  pairs,  $p=0.25$ ; NMDAR EPSCs - Untrans.:  $11.2 \pm 3.4$  pA, GluA1\_γ8 rescue:  $8.6 \pm 1.4$  pA,  $n = 5$  pairs,  $p=0.44$ ), and control and GluA1\_γ8 ΔPDZ rescued pairs (**c** - AMPAR EPSCs - Untrans.:  $-91.8 \pm 18.2$ , GluA1\_γ8 ΔPDZ:  $-8.5 \pm 2.0$ ,  $n = 9$  pairs,  $p=0.0039$ ; NMDAR EPSCs - Untrans.:  $15.5 \pm 3.7$  pA, GluA1\_γ8 ΔPDZ:  $10.0 \pm 2.4$  pA,  $n = 7$  pairs,  $p=0.031$ ). All statistics performed with a Wilcoxon matched-pairs signed rank test, filled circles represent mean  $\pm$  SEM. Source data are provided as a Source Data file.

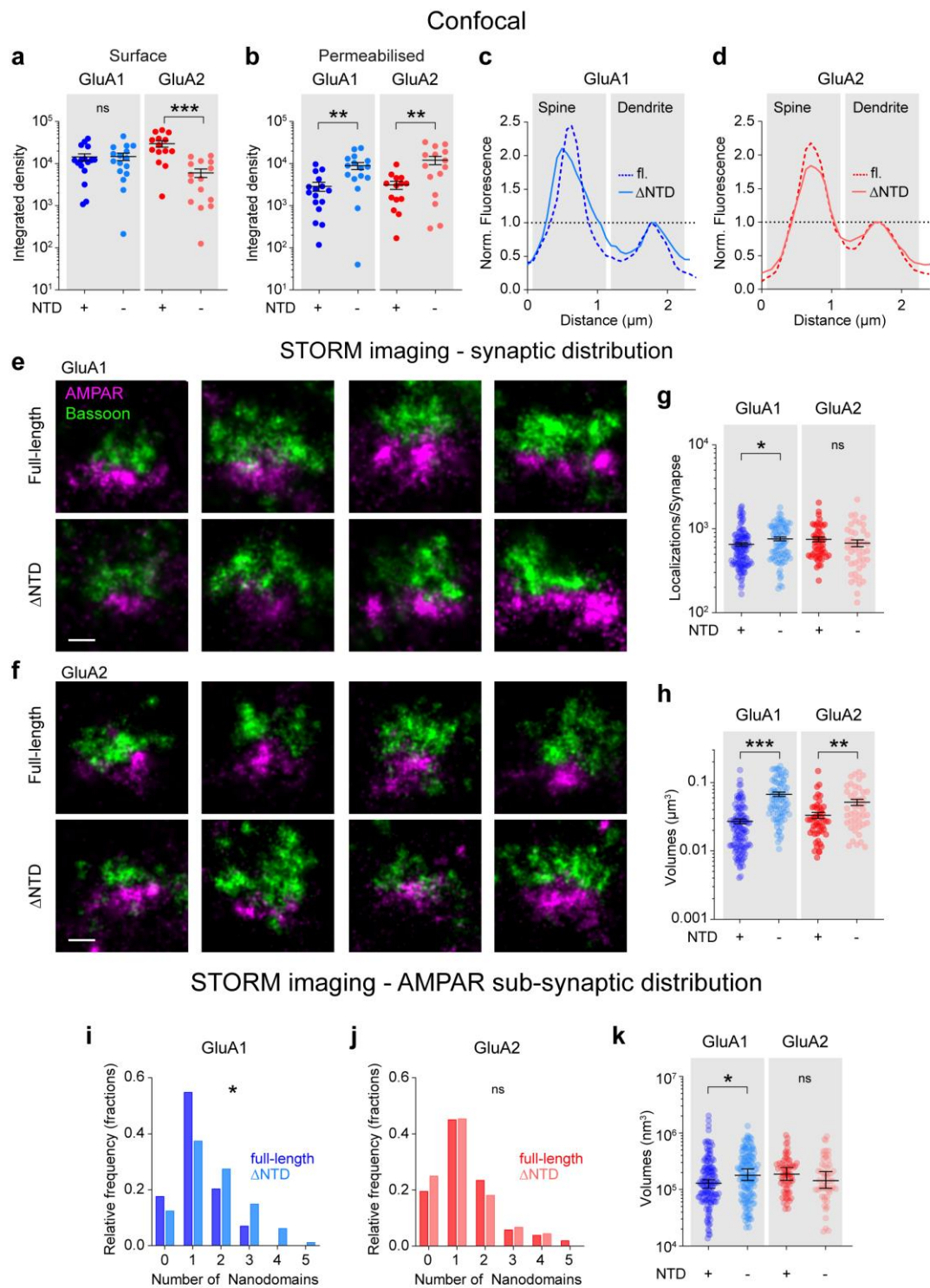


**Supplementary Figure 5. Supporting figures for knockout and rescue using GluA2\_γ8 or heteromeric AMPARs.** **a** Synaptic NMDAR currents are unchanged after AMPAR knockout and rescue with different GluA2Q\_γ8 constructs (GluA2Q\_γ8 - Untrans.:  $43.4 \pm 17.3$  pA, Rescue:  $22.5 \pm 6.4$  pA,  $n = 7$  pairs,  $p=0.16$ ; GluA2Q\_γ8 ΔPDZ - Untrans.:  $44.6 \pm 10.0$  pA, Rescue:  $50.8 \pm 13.2$  pA,  $n = 9$  pairs,  $p=0.25$ ; GluA2Q\_γ8 ΔPDZ ΔNTD - Untrans.:  $48.9 \pm 11.1$  pA, Rescue:  $46.8 \pm 6.1$  pA,  $n = 5$  pairs,  $p>0.99$ , All tests are Wilcoxon matched-pairs signed rank tests). **b** Synaptic NMDAR currents are unchanged after AMPAR knockout and rescue with different tandem heteromeric receptors (A1\_γ8/A2\_γ8 - Untrans.:  $36.7 \pm 4.1$  pA, Rescue:  $32.7 \pm 2.9$  pA,  $n = 14$  pairs,  $p=0.76$ ; A1\_γ8/A2\_γ8 ΔPDZs - Untrans.:  $83.9 \pm 9.7$  pA, Rescue:  $81.0 \pm 12.8$  pA,  $n = 14$  pairs,  $p=0.67$ ; A1\_γ8/A2<sub>A1NTD</sub>\_γ8 ΔPDZs - Untrans.:  $39.8 \pm 15.2$  pA, rescue:  $34.7 \pm 9.3$  pA,  $n = 6$  pairs,  $p>0.99$ , All tests are Wilcoxon matched-pairs signed rank tests). **c** Somatic AMPAR currents from outside-out patches on heteromeric receptor rescue. left -

Normalised current traces from holding potential ramp application to patches in the presence of glutamate, demonstrating non-rectifying responses on heteromeric receptor rescue. Both cell-averaged responses (bold) and individual cells (transparent) are shown (untransfected cells - grey, A1\_γ8/A2\_γ8 rescue - purple, A1\_γ8/A2\_γ8 ΔPDZs rescue - pink) against strongly rectifying GluA2Q overexpressing cell responses (black) for reference. right - Current amplitudes from somatic patches demonstrate abolishment of AMPAR response on Cre transduction, and similar surface receptor rescue by heteromeric receptors with and without TARP PDZ interactions (Untrans.:  $-434 \pm 118$  pA, n = 6; Cre:  $-20 \pm 6.5$  pA, n = 7; A1\_γ8/A2\_γ8 rescue:  $-456 \pm 74$  pA, n = 4; A1\_γ8/A2\_γ8 ΔPDZs rescue:  $-515 \pm 155$  pA, n = 5; one-way ANOVA with Dunnett' multiple comparisons test [ $F(3,18) = 6.007$ ,  $p=0.0051$ ]). \* indicates  $p<0.05$ . **d** NMDAR currents are unchanged on rescue with GluA1<sub>A2NTD</sub>\_γ8 ΔPDZs (Untrans.:  $46.7 \pm 9.4$  pA, Rescue:  $52.3 \pm 14.5$ , n = 11 pairs,  $p=0.96$ , Wilcoxon matched-pairs signed rank test). All data shown as mean  $\pm$  SEM. Source data are provided as a Source Data file.



**Supplementary Figure 6. Additional images supporting Figure 4.** Further representative images of surface AMPARs cell distribution from individual cells on GluA1\_γ8 rescue ± PDZ<sub>TARP</sub> interactions (cyan - tdTomato cell filler, red/glow - Streptavidin647 labelled surface AMPARs). For details of cell numbers and repetitions see Figure 4. Dendrite scale bars = 4 μm.



**Supplementary Figure 7. Supporting data for confocal and STORM imaging of cultured hippocampal neurons.** **a** Surface expression (confocal imaging) was unaltered for GluA1  $\Delta$ NTD receptors (integrated densities, left, GluA1 full-length:  $14410 \pm 2817$ , 16 cells, GluA1  $\Delta$ NTD:  $14880 \pm 2891$ ; 16 cells, 5 independent preparations;  $p=0.9080$ , two-tailed unpaired t-test) but significantly reduced for GluA2  $\Delta$ NTD receptors (integrated densities, right GluA2 full-length:  $29855 \pm 5081$ , 14 cells,

GluA2  $\Delta$ NTD:  $6072 \pm 1360$ ; 15 cells, 4 independent preparations;  $p < 0.0001$ , two-tailed unpaired t-test) compared to respective full-length controls. **b** NTD deletion resulted in increased intracellular expression levels for both GluA1 (integrated densities, left, GluA1 full-length:  $4007 \pm 956.1$ , GluA1  $\Delta$ NTD:  $12403 \pm 2352$ ; n-numbers given in a;  $p = 0.0025$ , two-tailed unpaired t-test) and GluA2 (right, GluA2 full-length:  $4295 \pm 923.4$ , GluA2  $\Delta$ NTD:  $16793 \pm 3806$ ; n-numbers given in a;  $p = 0.0046$ , two-tailed unpaired t-test) compared to full-length receptors. **c-d** Representative line-scans across spines and dendrites for full-length and NTD-deleted GluA1 (**c**) and GluA2 (**d**) receptors (See **Figure 5**). **e-f** Representative 3D STORM images to demonstrate synaptic distributions of presynaptic marker bassoon (green) and post-synaptic GluA1 (**e**) and GluA2 (**f**) full-length and  $\Delta$ NTD receptors (magenta) (Scale bars: 100 nm). **g** NTD deleted GluA1 (left, GluA1 full-length:  $647.5 \pm 33.8$ , GluA1  $\Delta$ NTD  $760.6 \pm 39.5$ ;  $p = 0.0190$ , two-tailed unpaired t-test) but not  $\Delta$ NTD GluA2 receptors (right, GluA2 full-length:  $749.9 \pm 50.4$ , GluA2  $\Delta$ NTD:  $675.4 \pm 64.9$ ;  $p = 0.0838$ , two-tailed unpaired t-test) displayed more localisations per synapse upon 3D STORM imaging. **h** NTD deleted GluA1 and GluA2 receptors adopted larger synaptic volumes compared to respective full-length receptors (synaptic volumes ( $\mu\text{m}^3$ ) left, GluA1 full-length:  $0.027 \pm 0.002$ , GluA1  $\Delta$ NTD  $0.067 \pm 0.005$ ;  $p < 0.0001$ , two-tailed unpaired t-test; (right, GluA2 full-length:  $0.033 \pm 0.003$ , GluA2  $\Delta$ NTD:  $0.052 \pm 0.005$ ;  $p = 0.0038$ , two-tailed unpaired t-test). **i-j** Relative frequency distribution of nanocluster numbers across individual synapses was changed in GluA1  $\Delta$ NTD receptors (left,  $p = 0.017$ , Kolmogorov-Smirnov test) but unaffected in NTD-lacking GluA2 receptors (right,  $p > 0.999$ , Kolmogorov-Smirnov test). **k** NTD deleted GluA1 (nanodomain volumes ( $\text{nm}^3$ ) left, GluA1 full-length:  $1.30 \times 10^5 \pm 1.06 \times 10^5$ - $1.50 \times 10^5$ , GluA1  $\Delta$ NTD  $1.79 \times 10^5 \pm 1.45 \times 10^5$ - $2.33 \times 10^5$ ;  $p = 0.0307$ , two-tailed Mann-Whitney test) but not  $\Delta$ NTD GluA2 receptors (right, GluA2 full-length:  $1.88 \times 10^5 \pm 1.45 \times 10^5$ - $2.46 \times 10^5$ , GluA2  $\Delta$ NTD:  $1.44 \times 10^5 \pm 1.05 \times 10^5$ - $2.09 \times 10^5$ ;  $p = 0.2257$ , two-tailed Mann-Whitney test) displayed larger nanodomain volumes. Data in panels a, b, g and h shown as mean  $\pm$  SEM and in panel k as median  $\pm$  lower-upper 95% CI, \* indicates  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  and <sup>ns</sup> specifies no significance. n-numbers for STORM data are given in legend to **Figures 5&6**. Source data are provided as a Source Data file.